Permeability of the Blood-Brain Barrier for Dilept and Its Active Metabolite

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Experiments on rats with measurements by HPLC-MS/MS showed that antipsychotic preparation dilept (N-caproyl-L-prolyl-L-tyrosine methyl ester) administered *per os* in doses of 40 and 200 mg/kg crossed the blood-brain barrier and was detected in rat brain (unchanged drug and its active metabolite N-caproyl-L-prolyl-L-tyrosine). The brain/plasma distribution coefficient for dilept was 2.0, for metabolite 0.5. No dose-concentration relationship was found, presumably because of the high dose of the drug transported from the blood to the brain not only by free diffusion, but also with participation of active carriers, whose number was limited.

Key Words: *blood-brain barrier; dilept; dilept metabolite; peptide carriers*

Dilept, a new drug of dipeptide structure (N-caproyl-L-prolyl-L-tyrosine methyl ester) has been created at V. V. Zakusov Institute of Pharmacology. The drug exhibited antipsychotic effects in experiments [2,5]. Because of metabolic instability and low bioavailability of neuropsychotropic drugs of peptidergic structure, it is essential to study their penetration through the blood-brain barrier (BBB) and delivery to the target organ (brain) for realization of their effects in CNS.

We studied BBB permeability for a new drug dilept (N-caproyl-L-prolyl-L-tyrosine methyl ester) and its active metabolite (N-caproyl-L-prolyl-L-tyrosine) after oral administration of dilept tablets.

MATERIALS AND METHODS

The study was carried out on outbred male albino rats (200±20 g, n=32) from Stolbovaya Breeding Center of the Russian Academy of Medical Sciences. The animals were kept under standard vivarium conditions at 12:12 day:night regimen with free access to water and food. Controls (n=8) received distilled water, experimental animals received oral dilept (mass prepared from tablets) in two doses: 40 and 200 mg/kg. The rats were decapitated by the standard method 15 min and 4 h after dilept administration. The first term was chosen as the time of presumable maximum concentration, the other as the time of presumable minimum concentration. Each group (for each dose and each period) consisted of 8 animals. The blood was collected into tubes with heparin, plasma was separated by centrifugation at 4000 rpm for 10 min at -4°C. Specimens of the plasma were prepared similarly as specimens of homogenated brain. All operations were carried out in the cold. The brain was washed in cold distilled water, dried with filter paper, weighed, and homogenized in a glass homogenizer for 1 min in distilled water (1:3 sample-water ratio). Proteins were precipitated ex tempore by adding double volume of acetonitrile with subsequent centrifugation at 4000 rpm for 10 min at -4°C. In order to separate the most hydrophobic compounds, and hexane (1:4 v/v) was added to deproteinized brain homogenate, the mixture was shaken (5 min), centrifuged for separation of the layers (4000 rpm, 5 min, 20°C), the hexane layer was discarded. Acetic acid (1 ml 0.7% solution) was added to 3.5 ml water homogenate. Extraction of dilept and its metabolite was carried out using 2.0 ml chloroform during 2-min shaking. After centrifugation (2500 rpm,

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5 min, 20°C) the organic layer was collected and dried in nitrogen flow. Dry residue was dissolved in 50 µl methanol and analyzed by HPLC-MS/MS (MS: mass spectrometry). Plasma and brain levels of dilept and its metabolite were measured by previously developed HPLC-MS/MS [1]. Penetration through BBB was evaluated by coefficient of distribution for the studied compounds ($K_{brain/plasma}$). The results were processed using Statistica 6.0 software.

RESULTS

The main physicochemical characteristics of dilept and its metabolite have been calculated on the basis of the structural formula of dilept molecule using the ACDDLABS-8 software [9]. By these estimated characteristics, penetration of the studied compounds through BBB can be predicted. According to the "rule of five", suggested by C. Lipinski [6], good absorption and permeability of a drug can be expected if its molecular weight is below 500 a.m.u., its octanol/water distribution coefficient LogP is below 5, number of hydrogen bond donors, expressed by the number of OH^- and NH^- groups, is less than 5, and number of hydrogen bond acceptors, (number of N^- and O^- atoms), is less than 10. It is assumed that if a substance does not conform to the "rule of five", it does not penetrate through BBB by free diffusion in physiologically significant amounts. The molecular weight of dilept is 390 a.m.u., octanol/water distribution coefficient LogP 2.51, number of hydrogen bond donors 2, and number of hydrogen bond acceptors 7. Hence, dilept conforms to Lipinski's rule and theoretically can penetrate through BBB by free diffusion. The molecule of dilept metabolite has similar parameters: molecular weight 376 a.m.u., the sum of hydrogen bond acceptors is the same as in dilept (7), number of hydrogen bond donors is 3, and LogP is 2.36, and at the same time, it is more polar in comparison with dilept, which can be essential for its transport through BBB. Many papers describe active transport of di- and tripeptide drugs through BBB with participation of peptide transporting systems and by means of endocytosis [3,4,7,8,10]. Discovery of PEPT1 and PEPT2 peptide carriers has become a potent stimulus for creation of new pharmacologically active peptide-based compounds and drugs [3,4,6,7,10]. The PEPT-1 is located mainly in the gastrointestinal tract and promotes active transport of intact di- and tripeptides through its membranes, increasing the absorption and bioavailability of peptide drugs. The PEPT2 is located in the brain, participates in active transport of small peptides through BBB, and delivers them to the target organ, the brain.

Dilept and its active metabolite are detected in the brain tissue of rats 15 min after oral administration of the drug tablet mass in two doses of 40 and 200 mg/kg (Tables 1,2). This indicates the permeability of BBB for these molecules. The $K_{brain/plasma}$ coefficient of distribution is higher for dilept (2.0) and is 0.5 for its active metabolite. Dilept and its metabolite remain for a long time (up to 4 h) in the brain.

TABLE 1. Dilept Content in the Plasma and Brain of Rats after Oral Administration of the Drug Tablet in Two Doses (*M±m*)

Time, min	Dose, mg/kg	Dilept content		Brain/plasma distribution coefficient
		plasma, ng/ml	brain ng/g tissue	
15	40	4.27±4.0	8.78±3.5	2.08
15	200	4.15±3.8	8.25±3.9	1.98
240	200	4.50±3.2	8.15±2.35	1.80

TABLE 2. Content of Dilept Active Metabolite in the Plasma and Brain of Rats after Oral Administration of the Drug Tablet in Two Doses (*M±m*)

Time, min	Dose, mg/kg	Dilept content		Brain/plasma distribution coefficient
		plasma, ng/ml	brain ng/ml tissue	
15	40	52.0±4.5	25.3±2.5	0.517
15	200	62.0±7.5	30.0±1.5	0.47
240	200	26.0±4.0	12.7±2.0	0.48

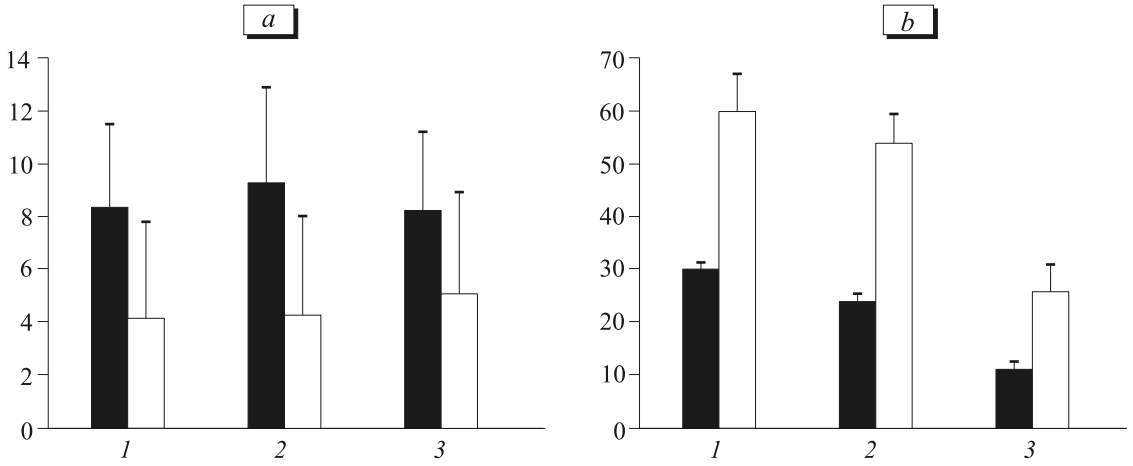

Fig. 1. Penetration of dilept (*a*) and its active metabolite N-caproyl-L-prolyl-L-tyrosine (*b*) through rat BBB. Dark bars: brain; light bars: plasma. Abscissa: dose (mg/kg) and time after drug administration (min): *1*) 200 mg/kg, 15 min; *2*) 40 mg/kg, 15 min; *3*) 200 mg/kg, 240 min. Ordinate: concentrations of dilept and its metabolite (ng/ml plasma and ng/g brain).

The concentration of active metabolite is higher than that of dilept, due to its higher concentration in the plasma (Fig. 1). No relationship between the dose and concentration was detected. The absence of the dose—concentration relationship can be explained by the double mechanism of dilept and its metabolite transport through BBB: participation of specific peptide transporters PEPT2, whose binding to the substrate is limited [10]. High value of the distribution coefficient for dilept indicates also that its transport can be realized by the free diffusion mechanism as well, which corresponds to theoretical prerequisites in accordance with Lipinski' rule.

Hence, the data on penetration of dilept and its active metabolite into the brain suggest the possibility of their direct effects on CNS and manifestation of the central effects.

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